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EFFECTS ON HONEYBEES OF SPRAYING FRUIT TREES WITH ARSENICALS

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INTRODUCTION

Ever since fruit trees have been sprayed with arsenicals beekeepers have been interested in the effect of this practice on the honeybee. Years ago, when sprayed materials were often applied while the trees were in full bloom, beekeepers became greatly excited and widely distributed literature entitled "Don't Spray While in Bloom." As a result of this agitation, according to Gates (*7*)[2] one Province in Canada and four States in the United States enacted laws prohibiting the spraying of fruit trees in full bloom. As late as December, 1920, there were laws to this effect in four States.

The subject of poisoning bees is very large and of vital interest, not only to beekeepers but to everyone who is interested directly or indirectly in the growing of crops, including particularly fruit growers, entomologists, and plant pathologists. Of course the beekeeper does not want his bees poisoned, chiefly because as a result his honey crop is reduced; but this loss is only secondary in comparison with the loss from lack of cross-pollination of flowers. In pollination the beekeeper, the fruit grower, and in fact everyone is benefited by honeybees. For several years evidence has been accumulating to prove beyond doubt that bees are extremely important as agents of pollination, and in fact should be protected and raised if only for this purpose. The honey they produce may be left for separate consideration.

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[1] Resigned June 30, 1923. The following men, who have also resigned from the Bureau of Entomology, had charge of the spraying in these experiments at their respective field stations: E. B. Blakeslee at Winchester, Va., 1914, 1915, and 1916; F. L. Simanton at Winthrop, Me., and at Benton Harbor, Mich., 1914 and 1915, respectively; and E. W. Geyer at Roswell, N. Mex., 1915. The junior writer did part of the work at Winchester, Va., 1915 and 1916, and at Fennville, Mich., 1916, and the senior writer did the remaining portion of the work here reported. Credit is due the Insecticide and Fungicide Laboratory, Bureau of Chemistry, for the chemical work reported in this bulletin.

[2] Reference is made by number (italic) to "Literature cited" p. 32.

The investigation here reported was begun in the spring of 1914 at the suggestion of Dr. A. L. Quaintance, in charge of fruit insect investigations, and was originally planned to determine the effect on honeybees of spraying fruit trees in full bloom. After one season's work in two States, it was ascertained that spraying at this stage under favorable conditions was detrimental to bees, and these results suggested that spraying even at the customary time, when at least 90 per cent of the petals have fallen, might be slightly injurious to them. This idea is in accord with the one which beekeepers often express by claiming theoretically that spraying fruit trees at any time is injurious to bees, the degrees of damage done depending on the time and on other conditions. Entomologists, however, do not recommend spraying orchards in full bloom (1) because the codling moth can be as well controlled by spraying when 90 per cent of the petals have fallen and (2) because spraying when the trees are in full bloom is injurious to insect pollinators.

After three seasons' work on this problem, in four States and five localities, it was determined that spraying at the customary time under nearly ideal conditions was not injurious to bees; but it will be noted that these nearly ideal conditions seldom occur.

Other projects were discussed and partially planned, but only one of these was finally investigated. The subject of the poisoning of bees by sprayed or dusted cover crops, often grown in orchards, is vitally important to beekeepers, but it seems that fruit growers pay little attention to this phase of the general subject of poisoning bees. This subject was only incidentally touched upon, as reported under the experiments performed at Roswell, N. Mex.

During the past few years it has become common to dust calcium arsenate or lead arsenate upon a large variety of plants, including fruit trees, flowering shrubs, fields of cotton, and even forest trees. This general practice of spreading poisons keeps the beekeepers constantly excited, although probably without warrant, and they have a feeling that their business may be doomed any day. Furthermore, it is now common in certain localities of the South to mix calcium arsenate with molasses and then to spray or to daub this poisonous mixture on cotton plants for the purpose of attracting and poisoning the boll weevil. As a part of this general project, the senior writer in July and August, 1922, carried on experiments at Tallulah, La., to determine the effects on honeybees of dusting cotton plants with calcium arsenate, and also of treating these plants with poisoned sweet mixtures. A summary of their results (*10*) has already been published, and, since there is no immediate prospect of doing more work along this general line, it is considered expedient to publish the result of the other, earlier work.

It is now generally admitted that in using arsenicals as a control for the codling moth the best results are in nearly all cases obtained by applying the first spray after most of the petals have fallen, although in commercial orchards where hundreds of acres of trees must be sprayed within a limited time it is necessary to begin spraying early in order to finish the work before the calyx cups close. Furthermore, in regions where the trees bloom irregularly, causing a long blooming period for an entire orchard, the trees sprayed vary from those in full bloom to those from which all the petals have fallen.

Many reports from beekeepers concerning the effect on the honeybee of spraying trees in full bloom have been published, but only a few systematic investigations have actually been carried on to determine whether or not the practice is injurious to bees. Some beekeepers believe that the honeybee is damaged slightly, even when the trees are sprayed at the customary time, after at least 90 per cent of the petals have dropped. No careful observations have been reported on this phase of the subject.

In the hope of reaching definite answers to some of these questions, so long debated, the writers planned and conducted the research here reported. Investigations, extending from 1914 to 1917, were made along three lines: (1) The effect on honeybees of spraying fruit trees in full bloom; (2) the effect on honeybees of spraying the trees at the customary time, after most of the petals have fallen; and (3) a determination of the minimum amount of arsenic required to kill bees in confinement.

EFFECT ON HONEYBEES OF SPRAYING FRUIT TREES IN FULL BLOOM

PRELIMINARY EXPERIMENTS, WINCHESTER, VA., 1914

Three small, more or less isolated orchards in full bloom, lying southeast of Winchester, Va., were selected in 1914 for the preliminary experiments. Three strong colonies of bees were installed in each orchard. Twenty-five trees in the first orchard were sprayed with a mixture of 5 ounces of Paris green, 1 gallon of limewater, and 49 gallons of water; 25 trees in the second orchard were sprayed with a mixture of 3 pounds of paste lead arsenate, 1 gallon of limewater and 49 gallons of water; and 25 trees in the third were sprayed with a mixture of 3 pounds of paste lead arsenate, 1.5 gallons of lime-sulphur and enough water to make 50 gallons in all.

As a result of these preliminary experiments the following points were ascertained: (1) Bees work equally as well on trees sprayed in full bloom as on unsprayed ones; (2) they do not fly away from the sprayed orchard very much if the orchard is well isolated; (3) they are slightly affected when a small orchard is sprayed in full bloom; this conclusion is supported by observations and by the analyses of the samples collected; and (4) of the three mixtures named, the lime-sulphur spray mixture is most satisfactory for experimental purposes, because the lime-sulphur in it serves well as a stain, so that the mixture can be easily seen on the foliage and blossoms. Its presence assures the observer that he is dealing with sprayed material when collecting blossoms for analyses and when watching bees at work on the supposedly sprayed flowers.

MORE EXTENDED EXPERIMENTS, WINTHROP, ME., 1914

It seemed desirable to continue on a larger scale the experimental work with the spray of lime-sulphur and lead arsenate. A large isolated commercial orchard in full bloom, almost ideally located three-fourths of a mile east of Winthrop, Me., was selected for that purpose. The orchard, consisting of 700 apple trees, was rectangular in form, bounded on the north by a 4-year-old orchard and a pasture, on the west by a pasture, on the south by cleared land and a 2-year-old orchard, on the east by a thicket, a lake, and a forest, and on the northeast by forest. There was a small orchard one-half mile north of this one and several commercial orchards one-half mile west of Winthrop. No other orchards were within sight of the one described.

Since only two small 7-frame hives of bees could be had at Winthrop, eight strong colonies belonging to the Bureau of Entomology were sent there by express from Philadelphia, Pa. All 10 colonies were free from brood disease.

APPLICATION OF SPRAY MIXTURE

Owing to a delay in the departure of the bees from Philadelphia, the spraying was begun before the bees were installed in the orchard. On May 25 and 26 Mr. Hinds, the owner of these trees, wishing to kill certain caterpillars, sprayed his entire orchard with a mixture of 4 pounds of paste lead arsenate and 1 quart of lime-sulphur to each 50 gallons of water, applying it with a 200-gallon power sprayer. On May 28 and 29, 310 of the trees, then in full bloom, were again sprayed, this time with a mixture composed of 3 pounds of paste lead arsenate and 1 pint of lime-sulphur to each 50 gallons of water

To kill the larvæ of the codling moth, *Carpocapsa pomonella* L., Mr. Hinds again sprayed the entire orchard on June 3, 5, and 6, using 3 pounds of paste lead arsenate and 1 gallon of lime-sulphur to each 50 gallons of water.

INSTALLATION OF BEES AND APPARATUS

The two colonies of bees procured at Winthrop were moved on May 29 from Mr. Wentworth's apiary, three-fourths of a mile northwest of that town, to the sprayed orchard. So far as could be determined none of the bees returned to their original location. The eight colonies from Philadelphia left there in the afternoon of May 27 and arrived at Winthrop late in the afternoon of May 29. They were installed in the orchard the following morning; but, owing to unfavorable weather, the bees did not have a good flight until 2 o'clock that day, after having been confined to the hives about 91 hours.

The 10 hives were placed between rows of sprayed trees at the south side, near the center of the orchard. Two sheets, each 7 yards square, were placed beneath two large sprayed trees, to catch the dead bees that fell from them.

To catch most of the dead bees removed from the hives a bee trap was devised, constructed as follows: A rectangular framework, 30 by 16 inches, was made of wooden strips 1½ inches wide by seven-eighths inch thick. The lower side (bottom of the trap) of the framework was covered with cheesecloth, and parallel pieces of twine one-fourth inch apart were stretched lengthwise over the upper side, seven-eighths inch above the cheesecloth. One side of the framework was nailed to the lower side of the alighting board, so that the framework projected 7 inches on either side of the hive. The sides of the trap were made of glass, 8 inches high, firmly fastened to the framework, and so snugly joined at the corners and where they united with the sides of the hive that a bee could escape only by going over the glass. A worker removing a dead bee from inside the hive usually flew from the alighting board with its load, but seldom flew sufficiently high to pass above the sides of the trap. Workers carrying dead bees therefore flew against the sides of the trap and fell to the bottom, where the hairs of the dead bees became fastened either to the cheesecloth or to the twine. The live bees then pulled themselves loose from the dead ones. One of these traps was placed in front of each hive entrance.

DAILY OBSERVATIONS OF BEES AND WEATHER

Notes pertaining to the bees and weather were carefully made, but only a brief report of the daily observations will be given here.

During the early morning of May 29 the two Wentworth colonies were installed; it was a clear, cool day, and the bees worked well on the sprayed blossoms. In the morning of May 30 the eight bureau colonies were installed, but as the weather was cool and rainy the bees did not leave their hives till 2 o'clock that afternoon. By this time the sun was shining brightly, the weather became warmer, and the bees, particularly the young ones, came in great numbers out of the hives. They flew to the nearest trees and began immediately to drink the water standing on the sprayed leaves and petals. Later the field bees were abundant in the trees and eagerly collected nectar and pollen. May 31 and June 1 were warm and cloudy, with occasional showers, although the bees worked well in the trees. June 2 was a cloudy, cool, and windy day, and the bees flew very little. Most of the petals had fallen, and many sick bees were seen in the bee traps and in the grass near the hives. June 3 was cool and clear, and the bees flew only fairly well during the middle of the day. June 4 was cool and rainy. One bee trap contained a half hatful of dead and dying bees. June 5 was a cool, clear and windy day, and practically all of the petals had fallen. Each trap contained many dead and dying bees. June 6 was a clear warm day; the bees flew well, and collected pollen from the wild flowers. Hundreds of dead and dying bees were seen in the traps and in the grass around the hives. June 7 was warm and cloudy, and the bees flew well. The bees were still dying rapidly; many were removed from the traps as usual and hundreds of sick ones lay in the grass, particularly to the leeward of the hives. On June 8 the bees were still dying to an abnormal extent; nevertheless, the colonies were prepared for shipment. The two Wentworth colonies were returned to their own apiary and the eight bureau colonies were expressed direct to Washington, D. C., where they were released June 11, 87 hours after having been closed. Nine pints of dead bees were removed from the eight hives, whereas only 3.75 pints had been taken from them at Winthrop upon their arrival from Philadelphia. It is safe to say that 5 of the 9 pints died on account of the poison, not taking into account the cooler weather and less crowded condition in favor of the bees in the hives during their journey to Washington.

In regard to the dead bees caught in the bee traps, it was found in the preliminary experiments that the average daily number per trap, counted before the trees had been sprayed, was 18, which represents about 3 per cent of all those that died, the 97 per cent dying in the field. The average daily number of dead bees per trap, counted in the traps at Winthrop after the trees had been sprayed, was 136. Evidently the bees in the sprayed orchard died more than seven times as rapidly as those in a normal environment. This ratio does not include the hundreds of sick bees that crawled out of the traps and died in the grass near the hives. If it had been possible to count all the bees that died from the effects of the poison and all those that died normally, the proportion of deaths from poisoning would certainly have been many times higher.

The unusual mortality of the bees was first noticeable the second day after they had had access to the sprayed flowers; on the third day after they had been poisoned the damage was unmistakable. This heavy mortality continued as long as the sprayed flowers lasted, and in some of the colonies did not cease until all the bees had died. Most of the sick and dead bees appeared to be young, and many of them bore wax scales. The field bees seemed to have died in the fields as usual, although only a few of them were found dead daily on the sheets under the sprayed trees. Under normal conditions the few field bees that die in the hives represent only a small percentage of the total number of the dead. Most of the dead and sick bees had swollen abdomens and often when an abdomen was gently squeezed a thick yellowish liquid was discharged from the anus. Most of the sick bees clustered for a short time in the traps, and later many of them climbed to the tops of the traps, where, after perching a few moments, they attempted to fly, but every bee observed fell into the grass only a few feet from the hive. During the later stages of arsenic poisoning a sick bee was usually drowsy and stupid, but often acted "crazily" by continuously turning around; then one or more legs became paralyzed, and the bee could walk only in a staggering manner, and could not fly; a little later it became paralyzed to such a degree that it could not get upon its feet, but could yet crawl about by dragging its swollen abdomen; it finally died in a state of coma.

When installed in the orchard 1 hive of bees was weak, 8 were strong, and 1 very strong. When transferred from the orchard 1 colony was slightly weaker than when it was brought there, 1 was weak, 1 strong, and 7 slightly less strong than when placed in the orchard. On June 15 the 2 Wentworth colonies experimented with were dying rapidly; on June 25 they were nearly depopulated, and on July 27 entirely so; and 1 of Mr. Wentworth's other 2 colonies (not experimented with) was nearly depopulated. The bees from the latter colony probably visted the sprayed orchards, for it was only 1½ miles distant. In regard to the 8 bureau colonies, on June

23 2 colonies were about as strong as when they left Maine, 4 were weak, 1 was nearly depopulated, and 1 entirely so. On July 3 one other colony was entirely depopulated; on August 1, one more; on September 14, three were fairly strong and 2 were weak. Not 1 of the 10 colonies used was killed outright, but 5 of them in a comparatively short time were entirely depopulated, while the other 5 were rendered more or less weak.

In the preliminary experiments the bees were installed in the orchards before the spraying had been done and were therefore working actively in the trees when the mixtures were applied. In most cases the bees were wetted by the sprays, by which they were driven from the trees, but returned in a few moments and continued to collect nectar and pollen. The lime-sulphur was the only substance used really repellent to the bees, but it did not keep them from the sprayed trees. In all cases they returned to the sprayed flowers before the lime-sulphur-arsenate mixtures on the foliage and blossoms had become entirely dry.

Immediately after the application of the spray mixtures, in the preliminary experiments, many sprayed flowers were closely examined. Most of them were thoroughly wetted with the sprays. Sometimes the cavities containing the stamens and pistils were completely filled with the mixtures, but never was any of the spray seen in the nectaries. These observations were repeated at Winthrop after applying the already-mentioned mixture of 3 pounds of paste lead arsenate to each 50 gallons of water in a 200-gallon power sprayer. In one tank of mixture 4 gallons of lime-sulphur was used; in another tankful one-fourth ounce of methylviolet was dissolved and a third tankful contained 3 ounces of an aqueous solution of eosin. The object of the dyes was to determine whether or not they would color the flowers better than the lime-sulphur, so that the presence of the spraying materials might be traced in the blossoms. They proved no better than the lime-sulphur for this purpose, but all three substances were fairly satisfactory for these tests. It was common to see the spray mixtures in the interior of the flowers, but they were never traced into the nectaries; the analyses of the nectaries from fresh flowers (Table 1), however, indicated that the mixtures did pass into the nectaries. Fine spray was generally seen on the anthers and stigmas; and, after it had evaporated, the petals were usually covered with small brown, violet, or pink dots, but the inner surfaces of the stamen bars at or near their bases never appeared to be stained.

On several occasions bees were closely observed when collecting pollen and nectar. Several bees with more or less pollen on their hind legs were seen collecting only nectar, but most of those with pollen on their hind legs were taking only pollen, while those without pollen on their hind legs were collecting only nectar. A bee collecting nectar usually alighted on the bunch of anthers and stigmas and instantly thrust its mouth parts between the stamen bars in order to reach the nectar. The anthers carrying the pollen came in contact with the upper portions of the front legs and with the throat of the bee, and some of the pollen adhered to the many branched hairs on the parts touched.

A bee collecting pollen also usually alighted on the bunch of anthers and stigmas. It removed the pollen from the anthers with its mouth parts, and, in transferring the pollen to the pollen baskets by means of the legs, much of the pollen adhered to the hairs on all the legs. A few bees were seen completely covered with pollen dust. When a bee returns to the hive with its load of nectar or pollen, it is usually cleaned before it leaves the hive again, most of the pollen dust adhering to the hairs being removed. The cleaners certainly eat some of the pollen dust removed, for bees in an observation hive are often seen to steal pollen from the pollen baskets before the bee carrying the pollen has placed its burden in a cell. Bees collecting the pollen probably swallow a small portion of the grains while biting them loose from the anthers.

RESULTS OF CHEMICAL ANALYSES

To ascertain whether chemical analyses would corroborate the results obtained by observation, many samples were collected on various dates, consisting of parts of sprayed flowers, fresh pollen from legs of bees and from combs, honey, entire bees, foreign substances removed from bees, parts of bees, dead pupæ, and dead larvæ. The results of analyses of these samples are briefly summarized in Table 1, which, with the exception of the last column, is self-explanatory. In that column the weight of arsenic found is expressed as parts per million in terms of metallic arsenic (As). For illustration, let us consider a sample containing flowers without the anthers and stigmas. The entire sample weighed 6.031 grams (about 0.21 ounce) and the metallic arsenic found in it weighed 0.1 milligram (about 0.0000035 ounce). One tenth of a milligram is found by a simple computation to be 16.6 millionths of 6.031 grams. The weight of arsenic found in the sample is therefore 16.6 millionths, or parts per million, of the weight of the sample itself.

Of all the parts of the flowers analyzed the pollen bore the most arsenic. Expressed as parts per million (using only whole numbers), pollen from the legs of bees bore 117 (Table 1); fresh pollen stored in the hives, 96; anthers and stigmas of sprayed flowers, 55; and sprayed flowers, not including the anthers and stigmas, which had been cut off, 32.

Of the 12 samples of honey analyzed only 1 contained arsenic, and that only a trace. Most of this honey was probably collected before the bees had been installed in the sprayed orchard. Entirely unripened honey was scarce, perhaps owing to the fact that the bees ate the honey almost as rapidly as it was collected. In the preliminary experiments 18 samples of more or less unripened honey were collected, but when analyzed not 1 of them was found to contain arsenic.

Nectaries carefully dissected from flowers sprayed in full bloom bore 0.0003 milligram each of arsenic, on an average (Table 1); nectaries from other flowers, sprayed after the petals had fallen, bore each 0.0007 milligram of arsenic. In computing these averages and others in Table 1 only the samples containing arsenic (As) were considered.

TABLE 1.—*Results obtained from chemical analyses of samples collected at Winthrop, Me., 1914, after trees in full bloom had been sprayed*

Material analyzed	Number of samples analyzed—		Average weight or number[1]	Average amount of arsenic (As) per individual in samples showing arsenic	Parts of arsenic (As) per million
	Showing arsenic	Showing no arsenic			
			Grams	*Milligrams*	
Sprayed flowers, after removal of anthers and stigmas	5	0	4.24	--------	32
Anthers and stigmas of the flowers	6	0	.40	--------	55
Pollen from legs of bees	4	0	.43	--------	117
Fresh pollen stored in hives	12	0	1.18	--------	96
Honey, partially ripe	1	11	7.40	--------	2
			Individuals		
Nectaries from flowers sprayed in full bloom	6	1	25	0.0003	--------
Nectaries from flowers sprayed after petals had fallen	8	2	25	.0007	--------
Dead bees from traps	15	4	37	.0004	
First wash, containing foreign matter from other bees from traps (no arsenic in subsequent washes)	1	3	95	.0002	--------
Nectar and pollen carriers	8	0	30	.0005	--------
Nectar and pollen carriers, after being washed five times	14	0	30	.0003	--------
Washings from 81 pollen carriers:					
First wash, containing foreign matter	1	0	81	.0003	--------
Second wash	1	0	81	.0001	--------
Third wash	1	0	81	.0002	--------
Fourth wash	1	0	81	.0001	--------
Fifth wash	1	0	81	.0001	--------
Nectar carriers, after removal of honey stomachs and intestines	4	0	25	.0002	--------
Honey stomachs of the nectar carriers	4	0	25	.0004	--------
Intestines of the nectar carriers	4	0	25	.0005	--------
Pollen carriers, after removal of honey stomachs and intestines	1	0	35	.0011	--------
Pollen carriers, washed five times, then honey stomachs and intestines removed	1	0	100	.0001	--------
Dead bees from traps, after removal of contents of abdomens	21	0	34	.0003	--------
Contents of abdomens of the bees	16	25	31	.0002	--------
Dead bees from traps, washed five times, then contents of abdomens removed	3	0	50	.0002	--------
Dead pupæ from traps	0	2	15	--------	--------
Dead larvæ from traps	0	2	50	--------	--------
Total number of samples analyzed	139	50	--------	--------	--------

[1] Average weight per individual of sample, or average number of individuals per sample, taken for analysis.

A large number of dead bees from the traps bore on an average 0.0004 milligram of arsenic each, including arsenic both within and without the body. To determine how much of this was carried outside the bodies, 95 other dead bees from the traps were washed four times in a 20 per cent solution of arsenic-free concentrated nitric acid and the fifth time in 95 per cent alcohol. These five liquids (here called washes), containing the foreign matter from the bees, were then analyzed; the first one contained 0.0002 milligram of arsenic per bee, the other four none.

Eight samples of live nectar and pollen carriers, averaging 30 bees each, caught on the alighting boards and killed in a cyanide bottle, carried, externally and internally, 0.0005 milligram of arsenic per bee, or 25 per cent more than was carried by the average of the dead bees from the traps. Fourteen other samples of live nectar and pollen carriers, averaging 30 bees each, caught and killed in the same manner, were washed five times, after which analysis showed that the bees still carried an average of 0.0003 milligram of arsenic per bee. The difference between these two averages, 0.0002 milligram per bee, agrees with the average (0.0002 milligram) removed from the 95 dead bees from the traps by five successive washes. Eighty-one pollen carriers, caught and killed in the same manner, were washed five times, and the successive washes analyzed; these contained per bee, in their consecutive order, 0.0003, 0.0001, 0.0002, 0.0001, and 0.0001 milligram of arsenic, respectively.

To trace the arsenic inside the bees, samples were analyzed as follows: Four samples of nectar carriers and one of pollen carriers, not washed, after removal of their honey stomachs and intestines, contained 0.0002 milligram and 0.0011 milligram, respectively, of arsenic per bee. The honey stomachs and intestines of the nectar carriers bore 0.0004 and 0.0005 milligram, respectively, of arsenic per bee. Pollen carriers, washed five times and deprived of their honey stomachs and intestines, averaged 0.0001 milligram of arsenic. Dead bees from the traps, after most of the abdominal contents had been squeezed out, averaged 0.0003 milligram of arsenic, while these contents carried 0.0002 milligram of arsenic per bee. Other dead bees, washed five times and similarly treated, averaged 0.0002 milligram of arsenic.

Two samples each of dead pupæ and dead larvæ were analyzed, but none of them were found to contain arsenic.

EFFECT ON HONEYBEES OF SPRAYING FRUIT TREES AT THE CUSTOMARY TIME

The experiments just described, performed at Winthrop, Me., in 1914, when the fruit trees were sprayed in full bloom, proved conclusively that the bees tested had been seriously injured; but since fruit trees are now seldom sprayed in full bloom, this knowledge does not have the economic significance that it would have had when spraying in full bloom was commonly practiced; nevertheless, there are other times, approaching the period of full bloom, when spray materials are commonly applied. In certain regions the time for the so-called pink application is often very short; accordingly some orchardists are still spraying when the blossoms burst open. In other regions the fruit trees of the same variety may bloom irregularly, and in still other localities an orchard often contains trees of different varieties, some blooming early and some late. Under such conditions an orchardist when spraying does not usually select the trees to be sprayed at the proper time, but sprays all as he approaches them, regardless of the age of their blossoms. That bees may be seriously damaged when the trees are sprayed in full bloom strongly indicates that they may also be injured under the conditions just mentioned, and it is furthermore suggested that bees might be slightly injured by spraying at the customary time, when 90 per cent of the petals have fallen.

To ascertain the effect on bees of spraying fruit trees at the customary time, the senior writer undertook a research at Roswell, N. Mex., in the spring of 1915, and later in the season repeated the experiments at Benton Harbor, Mich., using the dead-bee traps and analyzing samples of the dead bees collected. In the same season, at Winchester, Va., the junior writer undertook to determine the death rate of bees by daily weighing the colonies. Owing to unfavorable weather in all three localities, the results obtained were not conclusive, and the experiments were repeated in 1916 at Winchester and at Fennville, Mich., a town in the fruit belt of the Great Lakes, lying 6 miles from the eastern shore of Lake Michigan, by both writers working together, using both methods.

EXPERIMENTS AT ROSWELL, N. MEX., 1915

In regions where the irregular blooming of apple trees causes a long blooming period for an entire orchard, the trees to be sprayed range in condition from those in full bloom to those from which all the petals have fallen. Since it was desired to determine the damage done to honeybees by spraying trees in such regions, three commercial orchards, consisting of hundreds of acres of trees in an arid region about Roswell, N. Mex., were selected for experimental purposes.

On April 8, 1915, two apiaries totaling 105 colonies lying near these orchards were examined, and 10 dead-bee traps were installed. Daily observations of the bees were made as usual. From this date till April 23, when the first spray mixture was applied, bees in all the colonies, except the two numbered 3 and 8, were dying apparently at a normal rate. These two colonies suffered abnormal losses and exhibited symptoms of arsenic poisoning; and when 10 samples from them, each of 75 dead bees, were examined, all were found to contain arsenic, averaging 0.0003 milligram per bee. Eight samples of old pollen, stored in these two hives and probably collected from cover crops the preceding year, were also analyzed. Each one contained arsenic, averaging 9 parts per million.

On April 23, when on an average about 90 per cent of the petals had fallen, the spraying was begun. During the afternoon of April 24 a hailstorm stripped the trees of practically all the remaining petals, so that by April 26 every orchardist in the vicinity was beginning to spray. The spray mixture consisted of 2 pounds of paste lead arsenate to each 50 gallons of water, and was applied through three leads of hose under pressure varying from 200 to 240 pounds. Two 200-gallon power sprayers were used in all the orchards.

At regular intervals after the spraying was begun samples of dead bees were taken from each of the 10 traps. Of 8 samples taken from hives Nos. 3 and 8, which contained pollen stored in 1914, 7 contained arsenic, and of 16 samples from the other hives 15 contained arsenic in very small quantities. On April 28, when these experiments were discontinued, the 10 hives with traps were apparently as strong as when the work was begun, and no symptoms of arsenic poisoning were noticed except in the case of hives Nos. 3 and 8.

EXPERIMENTS AT BENTON HARBOR, MICH., 1915

Heavy rains and frequent hailstorms in the region about Roswell, N. Mex., made conditions there abnormal for experimenting with bees. As the blooming period in the fruit belt of the Great Lakes is comparatively long, it was decided to repeat at Benton Harbor, Mich., the experiments just described. On May 6, 1915, three small orchards and two apiaries of 35 colonies were selected there for this purpose. Ten dead-bee traps were installed and observations were made daily as usual. Owing to rainy weather and irregularity in the blooming of the different varieties of apple trees, spray mixtures of lead arsenate and lime-sulphur were applied whenever the weather permitted. As a result, a few trees were sprayed in nearly full bloom, several when 80 per cent and many when 90 per cent of their petals had dropped, and many after all of the petals had fallen.

Observations showed that a small percentage of the mortality of the bees was caused by arsenic poisoning, but none of the colonies was apparently reduced in number of bees. Thirty-three samples of dead bees were analyzed, of which 30 were found to contain arsenic in very small quantities.

EXPERIMENTS AT WINCHESTER, VA., 1915 AND 1916

The experiments conducted at Winchester in 1915, inconclusive in result because of unfavorable weather, were continued there in the spring of 1916 by both writers, using the dead-bee traps and analyzing samples of the dead bees collected, and also weighing certain colonies day by day. The latter method of investigation was first tried at Winchester in 1915. In both years the experiments were conducted in three apiaries, each free from foulbrood and surrounded by large commercial orchards: The Cooper apiary, of 70 colonies, lying 3 miles south of Winchester; the Miller apiary, of 40 colonies, 3 miles southwest; and the Lupton apiary, of 5 colonies, 2 miles west. For the weighing experiments in 1915 only the Cooper apiary was used, but in 1916 they were carried on in both the Cooper and Lupton apiaries. Six colonies, selected from the former apiary, were weighed daily, and the three following factors were considered in selecting them: (1) As a precaution against an abnormal death rate they were not transferred from their own apiary, nor were they unnecessarily disturbed; (2) they showed the best evidence of having had uninterrupted brood-rearing during the spring, and so of having a well-balanced ratio of old and young bees; and (3) they occupied well-marked positions in the apiary, thus preventing, so far as possible, errors due to the bees entering the wrong hive.

APPARATUS AND METHODS

Platform scales, sensitive to a variation of one-fourth ounce, were used to weigh the colonies. At Winchester these scales were located in a small building at one side of the apiary, and at Fennville, Mich., a temporary shelter was constructed for them. The primary purpose of the shelter, in each case, was to protect the scales from the wind and so prevent or reduce errors in the readings.

Chemical balances were used to determine the weight of the samples of a known number of bees and to ascertain the variation in the weight of the bees from day to day. In each case these balances were located in a building adjacent to the apiary.

In order to weigh the colonies when the bees were in the hive, the weighing was done in the morning, as soon as there was sufficient daylight for the work (about 4.30 o'clock). Each of the six hives containing the colonies under observation was in turn carried to the scales, weighed, the weight recorded, and the hive returned to its place in the apiary. After the gross weights of all hives, each with its contents, had been determined, the bees in each hive were quickly transferred to another, and the hive from which they were taken, together with its combs of brood and honey, was again weighed. The difference in the two weights was the weight of the bees, plus that of any honey they were able to take from the combs during the process of transferring. To prevent any addition to the population of the colonies by the emergence of young bees during the experiment, all combs containing nearly mature brood were removed from the hives as the brood approached maturity.

Since in this experiment the daily loss of individual bees was to be determined by the daily loss in weight of the colony, the variation in the quantity of honey that the bees might take from the combs and carry with them while being transferred would be a disturbing factor, but the method used in transferring them from one hive to another was such that they had no opportunity to take any honey from the combs, because they were kept moving from the time the hive was opened until they were in the other hive.

To shorten the necessary manipulation, only 5 combs instead of 10 were used in each hive. After the gross weights of all the hives had been determined hive No. 1 was removed from its stand and an extra hive with five empty combs but no cover was put in its place. Hive No. 1 was then lifted from its bottom board and set on top of the prepared hive. Its cover was removed, the five combs spread apart, and by means of a brush the bees were dislodged from the combs, while smoke was applied to prevent their return. Each comb was then removed, swept clean of bees, and placed in an empty hive body. Hive No. 1, now emptied and free from bees, was then reassembled and again weighed, with all parts, including the combs of brood and honey. This hive, with its five combs of brood and honey, then received colony No. 2, similarly transferred. This process was repeated until the weight of each of the hives and contents, minus the bees, was determined. The five combs of brood from colony No. 6 were then given to colony No. 1, and the empty combs which had been given temporarily to colony No. 1 when it was transferred were removed. This routine was repeated daily during the experiment, care being taken to finish the work before any bees could return from the fields with nectar, and so introduce error from that source.

To determine how much variation, if any, there was in the weight of individual bees from day to day, caused chiefly by their greater activity during the honey flow, a sample averaging 22.5 bees was weighed each day on the chemical balances. These bees were taken each morning from the same colony (No. 6) to avoid differences that might result from a difference of temperament. The bees were taken after having been dislodged from the combs to the hive from which they were transferred, and before they could reach the combs of the hive to which they were being transferred.

To obtain data on the collection of nectar from the fruit blossoms at various intervals during the day, the following methods were employed: To determine the load, largely of nectar, which the bees carried during the most favorable time for foraging, 10 outgoing and 10 returning field bees caught at the hive entrances were weighed on the chemical balances hourly during each day. To ascertain the increase, also consisting largely of nectar, of an undisturbed colony, a hive of bees was kept on the scales at Winchester in 1916, and hourly readings were made of the weight of the entire hive and contents. In 1916, at both Winchester and Fennville, the hives and combs of the colonies weighed daily were kept together in sets in order to obtain the daily gain or loss in weight. Furthermore, observations were made hourly during the day on the amount of visible nectar in the blossoms and the number of bees visiting them.

To determine the gain or loss of both old and emerging bees, two colonies (Nos. 17 and 18) in the Lupton orchard were weighed on the evenings of April 29, May 4, and May 11, after the bees had all returned to the hives. The weight of the bees, separate from the hive and combs, was found by a method similar to that used in the daily weight determinations previously described, but in this case the maturing brood was not removed.

Four dead-bee traps (Nos. 7 to 10) were installed in the Cooper apiary, four (Nos. 11 to 14) in the Miller apiary, and two (Nos. 15 and 16) in the Lupton apiary.

Data on the local temperature and humidity prevailing during the experiments were obtained by means of a thermograph, hygrograph, maximum and minimum thermometers, and a sling psychrometer. Observations were also made on the direction and velocity of the wind and character of day.

DAILY OBSERVATIONS

In 1915, at Winchester, abnormal weather conditions greatly hastened the development of the fruit blossoms. No weight records were obtained previous to the application of the spray mixture. On the morning of April 30 the first weight record (on the platform scales) was obtained, and spraying was begun in a large orchard 100 yards distant from the bees weighed. On April 29, when about 60 per cent of the petals had fallen, the bees worked freely on the blossoms; on April 30, after a strong wind and when 95 per cent or more of the petals had dropped, the bees worked less freely; and from this date till the end of the experiments on May 8 very little nectar was gathered from the sprayed blossoms.

On April 30 the total weight of the bees in the six hives weighed was 215 ounces (Table 2); but on May 8, eight days after the spraying was begun, the bees weighed 84 ounces less. The average daily loss was therefore 10.5 ounces, or 4.88 per cent of the initial weight. The loss in ounces for each of the several days from April 30 to May 8, inclusive, beginning with May 1, was 40, 15, 7, 5.5, 7, 1.5, 0.5, and 7.5 ounces. The first daily loss was exceedingly large; the others relatively small.

TABLE 2.—*Results obtained by weighing honeybees at Winchester, Va., 1915, to determine the effect on them of spraying fruit trees at the customary time*

Colony number	Weight of adult bees (after spraying) on the dates specified								
	April 30[1]	May 1	May 2	May 3	May 4	May 5	May 6	May 7	May 8
	Ounces	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*
1	43. 25	34. 00	32. 25	31. 75	30. 25	29. 50	29. 50	28. 50	26. 00
2	22. 00	17. 75	14. 00	13. 25	11. 50	10. 75	11. 25	10. 50	9. 25
3	40. 50	34. 00	33. 50	31. 00	30. 25	29. 00	27. 50	29. 50	27. 50
4	37. 75	31. 50	27. 25	27. 00	26. 50	26. 75	24. 50	23. 75	22. 75
5	30. 75	24. 75	23. 75	21. 25	21. 75	19. 00	19. 00	17. 75	18. 25
6	40. 75	33. 00	29. 25	28. 75	27. 25	25. 50	27. 25	28. 50	27. 25
Total weight	215. 00	175. 00	160. 00	153. 00	147. 50	140. 50	139. 00	138. 50	131. 00
Daily loss		40. 00	15. 00	7. 00	5. 50	7. 00	1. 50	0. 50	7. 50

[1] Date on which spraying was begun.

In 1916, at Winchester, from the time the trees were almost in full bloom till all the petals had dropped, the weather was almost ideal and the bees worked normally. Spraying was begun May 5, in the Lupton orchard; occasionally on that day a tree still having 50 per cent of its petals was sprayed, although most of the trees had lost more than 50 per cent but less than 90 per cent of their petals. On the same day a few bees were seen visiting these trees. On May 6 about 98 per cent of the petals had fallen from most of the trees, but a few trees still bore 10 per cent of their petals. A bee was occasionally seen visiting the large sprayed trees, but more were observed on a few young trees of a later variety which had also been sprayed. On May 7 a few bees were still observed visiting the sprayed trees; on May 8, when practically all the petals had fallen, only now and then was a bee seen in the trees.

On May 5 spraying was also begun 1¼ miles south of the Cooper apiary, but no spray was applied nearer this apiary until May 8. On the latter date, when more than 90 per cent of the petals had fallen, spraying was begun in the orchards near the Cooper apiary. On this date and during the three following days very few bees were seen in the trees in these orchards, but they were common on the sprayed dandelion and grape hyacinth under and between the fruit trees.

On May 8 spraying was begun 1 mile southwest of the Miller apiary and on May 10 it was general in the Miller neighborhood, but the Miller orchard was not sprayed till May 11. On that date no petals were left on the trees and no bees could be seen in the trees.

In regard to the dead bees counted in the bee traps before spraying, those caught in traps Nos. 7 to 10 averaged 35 a day; those in traps Nos. 11 to 14 also 35; and those in traps Nos. 15 and 16 only 17, making a general daily average of 31 dead bees per trap. After spraying was begun, the dead bees caught in traps Nos. 7 to 10 averaged 23 a day; those in traps Nos. 11 to 14, 19 a day; and those in traps Nos. 15 and 16, only 12 a day, making a general daily average of 19 dead bees per trap. It will be shown later that these figures represent only a small percentage of the mortality of the bees, but it is believed that they nevertheless represent a true mortality index of the colonies before and after the spraying was begun.

TABLE 3.—*Results obtained by weighing honeybees at Winchester, Va., 1916, to determine the effects on them of spraying fruit trees at the customary time*

Colony number	Weight of adult bees (before spraying) on the dates specified										Weight of adult bees (after spraying) on the dates specified				
	April 28	April 29	April 30	May 1	May 2	May 3	May 4	May 5	May 6	May 7	May 8[1]	May 9	May 10	May 11	May 12
	Ounces	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*
1	50.25	50.00	50.00	50.50	49.25	50.50	44.50	44.50	41.50	37.50	38.00	32.00	33.00	31.00	29.00
2	55.25	56.00	53.75	54.50	55.00	54.75	50.25	49.25	47.25	40.00	40.00	38.75	38.50	32.50	33.00
3	49.75	47.50	48.25	48.75	46.00	48.25	45.75	44.25	40.50	35.50	34.00	33.75	31.25	31.00	28.25
4	45.25	47.50	44.75	44.74	44.50	45.25	42.50	39.50	38.00	33.75	34.00	30.00	28.50	25.75	24.25
5	50.00	47.75	46.00	48.50	45.50	44.75	44.00	41.50	40.00	34.50	33.75	32.00	32.50	28.75	28.25
6	48.00	45.25	44.00	46.25	44.25	46.50	42.00	39.50	36.25	33.75	32.00	32.00	32.25	28.00	27.75
Total weight	298.50	294.00	286.75	293.25	284.50	290.00	269.00	258.25	243.50	215.00	211.75	198.50	196.00	177.00	170.50
Daily loss or gain		−4.50	−7.25	+6.50	−8.75	+5.50	−21.00	−10.75	−14.75	−28.50	−3.25	−13.25	−2.50	−19.00	−6.50
Average daily load per returning field bee	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram* 0.0510	*Gram* 0.0260	*Gram* 0.0170	*Gram* 0.0070	*Gram* 0.0070	*Gram* 0.0080	*Gram* 0.0020	*Gram*	*Gram*	*Gram*
Average daily weight per individual bee in colony No. 6	0.0968	0.0999	0.1094	0.1121	0.1195	0.1088	0.1120	0.1095	0.1079	0.1057	0.1185	0.1041	0.1110	0.1004	0.1098
Maximum temperature	61	72	78	86	86	81	74	82	84	80	87	78	78	79	
Minimum temperature	45	43	43	50	56	59	58	55	59	56	56	58	46	56	56
Character of day	Part cloudy.	Clear.	Clear.	Clear.	Part cloudy.	Part cloudy.	Part cloudy.	Clear.	Clear.	Showers	Part cloudy.	Clear.	Part cloudy.	Clear.	Clear.
Character and direction of wind	Light W.	Calm S.	Calm S.	Light S.	Light S.W.	Calm S.	Light N.	Light W.	Light W.	Calm S.W.	Light S.W.	Strong W.	Strong S.	Strong W.	Strong S.W.

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[1] Spraying begun on this date near the bees under observation.

On April 28, 1916, the total weight of the bees in the six hives weighed in the Cooper apiary at Winchester was 298.5 ounces (Table 3). The daily loss (or gain) in ounces from April 28 to May 12, inclusive, beginning with April 29, was 4.5, 7.25, 6.5 (gain), 8.75, 5.5 (gain), 21, 10.75, 14.75, 28.5, 3.25, 13.25, 2.5, 19, and 6.5 ounces. Reference to Table 3 shows slight daily losses, and on two days (May 1 and 3), slight gains, from April 28 to May 3, and heavier losses thereafter. Since none of the samples of dead bees collected before May 8 in this apiary contained arsenic when analyzed, it is reasonable to suppose that these bees had not visited the sprayed trees 1¼ miles south of their apiary. Before the weighed bees had visited the sprayed trees they lost 9.28 ounces as a daily average, being 3.11 per cent of their initial weight; after having visited the sprayed trees near by, they lost 8.9 ounces as a daily average, being 3 per cent of their initial weight. The theoretical daily mortality, based on six weeks as the normal life of bees, is 2.38 per cent.

RESULTS OF CHEMICAL ANALYSES

At Winchester, in 1915, 6 samples of dead bees, 9 samples of pollen, and 6 samples of honey were collected and analyzed. Not one was found to contain arsenic. In 1916, at Winchester, samples of dead bees and pollen were, as usual, collected and analyzed. Five of 18 samples, each of 100 dead bees, contained no arsenic; analysis of the others indicated the presence of arsenic, although not enough to have killed the bees analyzed (see pp. 24 to 26). Six of 10 samples of pollen analyzed contained no arsenic; the others had only a trace of it.

EXPERIMENTS AT FENNVILLE, MICH., 1916

The experiments just described were repeated in 1916 at Fennville, Mich. Three apiaries, surrounded by commercial orchards, were selected. The Langley apiary of 12 colonies lay 4½ miles west of Fennville, the Pratt apiary of 14 colonies 6 miles west and 100 yards from the lake shore, and the Pshea apiary of 30 colonies 5 miles northwest. For the weighing experiments 6 colonies in the Langley apiary were used.

APPARATUS AND OBSERVATIONS

The platform scales and chemical balances were installed in a temporary shelter by the side of the Langley apiary, and three dead-bee traps (Nos. 7 to 9) were also installed in front of hives Nos. 7 to 9 in this apiary. Four bee traps (Nos. 10 to 13) were installed in the Pratt apiary and four (Nos. 14 to 17) in the Pshea apiary.

From May 22 to June 2 (the period of the experiments) the weather was almost ideal (Table 4), the blossoms secreted nectar more abundantly than they did at Winchester, and the bees worked well in the trees. The number of bees visiting the trees during full bloom and later agreed closely with the number observed during the same period at Winchester.

On May 22, 24, and 25, two orchardists sprayed their fruit trees, nearly in full bloom, located 1 mile west and southwest of the Langley apiary, with lime-sulphur and lead arsenate. Daily observations showed that the trees were not visited by bees. On May 29 spraying was begun near the Langley apiary. Practically all the petals had fallen and the bees had almost ceased visiting the trees. On May 30 the orchards nearest the Langley and Pshea apiaries were sprayed, but no bees were observed in the trees. On May 31 spraying was general in the neighborhood of all the bees under observation, and spray mixture was applied thereafter for several days.

TABLE 4.—*Results obtained by weighing honeybees at Fennville, Mich., 1916, to determine effect on them of spraying fruit trees at the customary time*

Colony No.	Weight of adult bees (before spraying) on the dates specified					Weight of adult bees (after spraying) on the dates specified				
	May 24	May 25	May 26	May 27	May 28	May 29[1]	May 30	May 31	June 1	June 2
	Ounces	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*
1	71.50	65.50	63.25	60.00	55.25	50.00	50.50	48.50	44.75	45.00
2	53.75	49.25	50.00	43.50	46.00	38.00	37.00	35.50	31.50	31.00
3	92.25	89.00	88.00	81.50	77.25	68.75	68.50	64.75	59.25	58.25
4	82.25	74.50	78.00	73.00	69.00	64.00	61.75	61.00	52.00	50.75
5	34.75	33.00	34.75	28.75	30.00	26.25	26.00	24.50	22.50	19.00
6	65.75	59.00	56.00	51.25	50.25	44.00	44.75	42.00	36.00	34.75
Total weight	400.25	370.25	370.00	338.00	327.75	291.00	288.50	276.25	246.00	238.75
Daily loss	--------	30.00	.25	32.00	10.25	36.75	2.50	12.25	30.25	7.25
Average daily load per returning field bee	*Gram* 0.0412	*Gram* 0.0485	*Gram* 0.0529	*Gram* 0.0369	*Gram* 0.0316	*Gram* 0.0250	*Gram* 0.0149	*Gram* 0.0249	*Gram* 0.0209	*Gram* --------
Average daily weight per individual bee in colony No. 6	.1210	.1232	.1255	.1288	.1225	.1089	.1271	.1197	.1147	.1074
Maximum temperature	75	79	85	73	73	74	64	69	74	--------
Minimum temperature	55	62	62	62	51	59	53	45	52	62
Character of day	Part cloudy.	Clear.	Clear.	Part cloudy.	Clear.	Rainy.	Clear.	Clear.	Part cloudy.	Clear.
Character and direction of wind	Light W.	Strong SW.	Calm SW.	Strong N.	Light NW.	Light SW.	Strong N.	Calm NW.	Strong S.	Light SW.

[1] Spraying begun near the Langley apiary and continued for several days.

On the basis of the number of dead bees counted in the bee traps before spraying was begun, they died in hives Nos. 10 to 13 at the rate of 13, and in hives Nos. 14 to 17 at the rate of 12 per day, making an average of about 13; after spraying was begun they died in hives Nos. 10 to 13 at the rate of 8 and in hives Nos. 14 to 17 at the rate of 10 per day, an average of 9. Colonies Nos. 7 to 9 in the Langley apiary have not been considered in computing these figures, because both before and after the spraying was begun they did not behave normally, being weak and badly diseased, and one was queenless.

On May 24, 1916, the total weight of the bees in the six hives weighed in the Langley apiary at Fennville was 400.25 ounces (Table 4). The daily loss in ounces from May 24 to June 2, inclusive, beginning with May 25, was 30, 0.25, 32, 10.25, 36.75, 2.5, 12.25, 30.25, and 7.25 ounces. The apparent heavy losses on May 25 and 27 and June 1 occurred coincidentally with an interference in balancing the scales when a strong wind was blowing. The weight of the bees in the sample taken on May 29, when the weather was rainy, suggests that the apparent heavy loss on this date may have been due to the lighter loads carried by the bees at the time they were weighed.

Since none of the samples of dead bees collected before May 29 in this apiary contained when analyzed more than a trace of arsenic (the amount usually found in control samples) it is reasonable to suppose that they had not visited the trees sprayed almost in full bloom 1 mile from their apiary. Before the weighed bees had visited the sprayed trees they lost 18.12 ounces as a daily average, or 4.53 per cent of their initial weight; after having visited the sprayed trees they lost an average of 17.8 ounces per day, or 4.45 per cent of the same initial weight. The theoretical daily mortality is 2.38 per cent.

RESULTS OF CHEMICAL ANALYSES

Samples of dead bees and pollen were collected and analyzed as usual. Of 19 samples of dead bees analyzed, 10 yielded traces of arsenic, although not enough to have killed the bees (see p. 24 to 26). Of 9 samples of pollen analyzed, 2 contained no arsenic, 2 a trace of arsenic, and 5 arsenic in larger amounts although not enough to have killed the bees.

EXPERIMENTS AT DRUMMOND, MD., 1917

The experiments just described were repeated on a small scale in 1917 at Drummond, Md., where the bee culture laboratory of this bureau was then located. Observations extended from April 27 to June 9. On April 30 the 11 apple trees there available were in full bloom and the bees worked abundantly in them. On May 7, when about 90 per cent of the petals had fallen and several bees were still visiting the blossoms, these trees were sprayed. From this date till May 14, when practically all the petals had fallen, very few bees were seen visiting the sprayed blossoms, and during much of this interval the weather was unfavorable for flying. On May 9 and 10 bees were observed collecting brown pollen from sprayed apple blossoms from which the petals had fallen. On May 26 they began storing nectar; from June 2 to 9 there was a heavy honey flow, although not from apple blossoms.

On April 27 the total weight of the bees in the 6 hives weighed at Drummond was 519 ounces. The daily losses (or gains) in ounces from April 27 to May 12 inclusive, and on May 19, May 26, June 2, and June 9, beginning with April 28 as the first day and ending with June 9 as the 43d day (Table 5) were 34.75, 16, 9, 21.75, 0.75 (gain), 11.75, 3.5, 18.75, 19.75, 30.5 (gain), 40.5, 0.25, 5.75, 12.75, 18.5, 35, 27.5, 35.5, and 33 ounces. Before the weighed bees had visited the sprayed trees they were losing daily 14.94 ounces, on an average, or 2.88 per cent of their initial weight; after having visited the sprayed trees, they lost during the first 6 days 7.88 ounces as a daily average, which was 1.52 per cent of their initial weight, or they lost 1.01 per cent as a daily average for the full period of 34 days after the trees had been sprayed. The theoretical daily mortality, based on six weeks as the normal life of bees, is 2.38 per cent.

Samples of pollen and bees were collected and analyzed as before, but this time the dead-bee traps were not used. Twenty samples of pollen were analyzed, none of which was found to contain arsenic; 10 of these were taken from the hives before and 10 after the trees had been sprayed. None of the 10 samples of pollen carriers, collected on May 10 and 11 was, when analyzed, found to contain arsenic. Of 10 samples of dead bees collected on May 14 and 22 in front of the hives, arsenic was found in only 3, and these came from three colonies which apparently had been suffering for some time from bee paralysis. However, no arsenic was found in samples of pollen taken from two of the three hives.

Table 5.—*Results obtained by weighing honeybees at Drummond, Md., 1917, to determine the effect on them of spraying fruit trees at the customary time*

Colony No.	Weight of adult bees (before spraying) on the dates specified									
	Apr. 27	Apr. 28	Apr. 29	Apr. 30	May 1	May 2	May 3	May 4	May 5	May 6
	Ounces	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*
1	89. 00	84. 00	83. 75	81. 50	76. 00	75. 00	73. 00	73. 00	69. 50	66. 25
2	100. 50	89. 50	85. 75	87. 50	85. 50	89. 25	81. 50	82. 50	81. 25	78. 00
3	63. 50	55. 25	57. 00	54. 00	54. 00	53. 00	54. 00	51. 50	49. 50	47. 00
4	98. 00	97. 50	90. 00	91. 00	83. 00	84. 50	84. 25	83. 00	77. 00	73. 25
5	111. 00	108. 00	99. 75	96. 50	91. 50	90. 00	88. 25	87. 50	83. 25	77. 50
6	57. 00	50. 00	52. 00	48. 75	47. 50	46. 50	45. 50	45. 50	43. 75	42. 50
Total weight	519. 00	484. 25	468. 25	459. 25	437. 50	438. 25	426. 50	423. 00	404. 25	384. 50
Daily loss or gain	--------	−34. 75	−16. 00	−9. 00	−21. 75	+0. 75	−11. 75	−3. 50	−18. 75	−19. 75
Average daily weight per individual bee in colony No. 6	*Gram* --------	*Gram* 0. 1060	*Gram* 0. 0965	*Gram* 0. 0974	*Gram* 0. 1087	*Gram* 0. 0990	*Gram* 0. 0973	*Gram* 0. 0958	*Gram* 0. 0852	*Gram* 0. 0923

Colony No.	Weight of adult bees (after spraying) on the dates specified									
	May 7[1]	May 8	May 9	May 10	May 11	May 12	May 19	May 26	June 2[2]	June 9
	Ounces	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*
1	69. 00	61. 00	64. 00	63. 25	59. 50	55. 00	48. 00	49. 00	46. 00	45. 50
2	78. 50	74. 50	72. 00	73. 50	70. 25	65. 50	62. 75	56. 75	53. 00	44. 25
3	52. 25	47. 25	49. 00	45. 25	44. 50	43. 75	40. 50	36. 00	32. 25	25. 00
4	83. 00	71. 25	69. 00	71. 50	67. 00	65. 00	61. 50	53. 75	51. 50	40. 50
5	85. 00	77. 75	75. 00	72. 50	74. 00	69. 25	57. 50	49. 25	26. 00	31. 00
6	47. 25	42. 75	45. 25	42. 50	40. 50	38. 75	32. 00	30. 00	30. 50	20. 00
Total weight	415. 00	374. 50	374. 25	368. 50	355. 75	337. 25	302. 25	274. 75	239. 25	206. 25
Daily loss or gain	+30. 50	−40. 50	−0. 25	−5. 75	−12. 75	−18. 50	−35. 00	−27. 50	−35. 50	−33. 00
Average daily weight per individual bee in colony No. 6	*Gram* 0. 0953	*Gram* 0. 1131	*Gram* 0. 0910	*Gram* 0. 0893	*Gram* 0. 0992	*Gram* 0. 0922	*Gram* 0. 1092	*Gram* 0. 1030	*Gram* 0. 1390	*Gram* 0. 1242

[1] Eleven neighboring trees sprayed.

[2] Heavy honey flow from June 2 to June 9.

Table 6 is a summary of the results obtained by weighing bees at Winchester, Fennville, and Drummond. The daily losses in ounces, recorded in Tables 2 to 5, have been changed to percentages. For comparative purposes these percentages and the theoretical normal daily mortalities are represented in Table 6 as accumulated daily losses. The daily losses of the bees at either Fennville, Winchester (1916), or Drummond may be compared singly with the theoretical daily losses, or the average daily losses recorded for these three localities may be compared with the theoretical figures. Since no weight records were taken at Winchester in 1915 before the spraying was begun, the percentages for this locality after the spraying was begun are not averaged with those of the other three localities, but are merely given for general purposes of comparison.

Table 6 clearly shows that the spraying did not affect the daily mortalities of the bees experimented with.

TABLE 6.—*Summary of results obtained by weighing honeybees at Winchester, Va., 1915 and 1916; Fennville, Mich., 1916; and Drummond, Md., 1917*

		Number of days before spraying								
		9	8	7	6	5	4	3	2	1
Accumulated daily loss	Loss, as indicated by actual weights at—	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
	Fennville, 1916	-------	-------	-------	-------	-------	7.50	7.56	15.55	18.11
	Winchester, 1916	1.51	3.94	1.76	4.69	2.85	9.88	13.48	18.43	27.97
	Drummond, 1917	6.70	9.78	11.51	15.70	15.56	17.82	18.50	22.11	25.92
	Average	4.10	6.86	6.63	10.19	9.20	11.73	13.18	18.70	24.00
	Theoretical loss, based on 6 weeks as normal life of bees	2.38	4.76	7.14	9.52	11.90	14.29	16.67	19.05	21.43

		Number of days after spraying									
		1[1]	2	3	4	5	6	13	20	27	34
Accumulated daily loss	Loss, as indicated by actual weights at—	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
	Fennville, 1916	27.30	27.92	30.98	38.54	40.35	-------	-------	-------	-------	-------
	Winchester, 1916	29.06	33.50	34.34	40.70	42.88	-------	-------	-------	-------	-------
	Drummond, 1917	20.04	27.84	27.89	29.00	31.45	35.02	41.76	47.06	53.90	60.26
	Average	25.47	29.75	31.07	36.08	38.23	-------	-------	-------	-------	-------
	Theoretical loss, based on 6 weeks as normal life of bees	23.80	26.19	28.57	30.95	33.33	35.71	52.38	69.05	85.71	100.00
	Loss, as indicated by actual weights at Winchester, 1915	18.60	25.58	28.84	31.40	34.65	35.35	-------	-------	-------	-------

[1] Day on which spraying was done or begun.

INTERPRETATION OF RESULTS

It is to be expected that the bees carried increasing quantities in their honey sacs from day to day as the honey flow increased, owing to the beginning of such activities as wax secretion and the evaporation of nectar, and that they carried decreasing quantities from day to day as the honey flow declined toward its close. This is verified in a general way by the weights of samples of a known number of bees taken in colony No. 6 previous to, during, and after the honey flows (Tables 3 to 5). For this reason it is to be supposed that the apparent loss in number of bees, as indicated by the weights, is less than the actual loss during the beginning of the honey flow and greater than the actual loss at the close of the honey flow. This is verified by the weight records (Table 3) obtained at Winchester in 1916, previous to (April 27 to 29) and during the honey flow (April 30 to May 5), and after its close (May 6 to 12). Using the average weight (0.0968 gram) per individual bee in the hives on April 28, just before the honey flow started, the average weight (0.1195 gram) on May 2, at the height of the honey flow, and the average weight (0.1098 gram) on May 12, after the close of the honey flow, it follows that, since there are 28.350 grams per avoirdupois ounce, an ounce of bees on the first date contained 292.9 bees; on the second, 237.2 bees; and on the third, 258.2 bees. The general average per bee of the 1,170 individual bees weighed in 52 samples at Winchester in 1915 and 1916 and at Fennville and Drummond is 0.1099 gram. Using this value, an ounce of bees is found to contain on an average 258 individual bees, or a pound would contain 4,128 bees.

At Winchester in 1915 (Table 2) and Fennville in 1916 (Table 4), the weights of the colonies were ascertained only during and after the honey flow from apple trees; it is therefore to be expected that the loss in number of bees as indicated by the weights at the close of the honey flow is, because of the decreasing quantities carried in their honey sacs, greater than the actual loss. This expected decrease is verified in the average weight of honey per bee in the samples taken at Fennville (Table 4); but, judging from the average weight per bee in the samples taken at Winchester in 1915, this decrease had already taken place before any samples were weighed, since none was weighed on the chemical balances in 1915 until May 2. These weighings are not published, as the season's work was brief and inconclusive.

During a 5-day period before the spraying, colony No. 17 in the Lupton apiary gained 12 ounces, or an average of 2.4 ounces per day; colony No. 18 gained 20 ounces, or 4 ounces per day. During a 7-day period after the spraying, colony No. 17 gained 18 ounces, or an average of $2\frac{4}{7}$ ounces per day; colony No. 18 gained 14 ounces, or 2 ounces per day. The total gain of 32 ounces for the two colonies both before and after the spraying was begun was certainly due to the addition of young bees to the colony, as the maturing brood had not been removed. It seems evident that the spraying had no effect on the combined weight of all the old and emerging bees; if the old bees were affected their decrease was balanced by an increase of the young ones.

The figures representing the nectar stored in the combs of the six hives weighed in the hive kept on the scales, and as a daily load per returning field bee, are all in harmony and their curves take the same general direction.

The weighing experiments showed that the death rate in a normal colony averaged about 600 bees per day, whereas the dead bees caught in the bee traps averaged only about 18 per day, thus indicating that about 97 per cent of the bees that died succumbed in the fields.

In summing up the results of the experiments conducted at Winchester and Fennville in 1916, and at Drummond in 1917, when the fruit trees were sprayed at the regular time, it appears for several reasons that the spraying did not injure the bees: (1) The daily weight records and the counts of dead bees in the traps show that the bees died more rapidly before than after the trees were sprayed. (2) The gain in weight of all the old and emerging bees before the spraying was begun was equal to the gain after, in the case of the two colonies tested. (3) After the spraying was begun the results obtained from the chemical analyses of the samples of dead bees did not indicate that the bees had died of arsenical poisoning. (4) No symptoms of arsenical poisoning were noticed at any time among the bees.

MINIMUM AMOUNT OF ARSENIC FATAL TO BEES IN CONFINEMENT

Chemical analyses of the samples of dead bees collected in 1914 at Winchester and Winthrop, after fruit trees had been sprayed in full bloom, showed that some samples taken at Winchester contained 0.0002 milligram of arsenic (As) per bee, and that some taken at Winthrop contained as much as 0.0004 milligram per bee. It was yet to be shown whether these quantities were fatal to bees. A series of experiments intended to decide this question was conducted at the bee culture laboratory at Drummond in July, August, and September of that year.

METHODS

To determine the behavior and to time accurately the longevity of bees fed definite quantities of arsenicals in confinement, 11 triangular observation cages were used, each made of 3 narrow wooden strips 1 inch wide, 2 of which were 10 and the third 6 inches long. Wire screen was used for the bottoms and tops of the cages, which lay on a table by a window. Fifty workers from alighting-boards were placed in each cage and fed definite quantities of poisoned candy or honey in small feeders, one to each cage, so covered with wire that the bees could not waste the food.

As an illustration of the method of procedure, 20 grams of finely pulverized confectioner's sugar and 1 milligram of finely pulverized Paris green were thoroughly mixed together; 20 grams of honey were added and the mixture again stirred thoroughly. The cages and feeders were carefully washed and dried to be sure that they were free of arsenic. One-tenth of this poisoned paste candy was put in each of 10 feeders, and nonpoisoned candy in the eleventh, for the control bees; in each cage were placed the 50 workers, and lastly the feeder containing the food, poisoned or otherwise. In a similar manner definite quantities of other arsenicals were thoroughly mixed with definite quantities of honey. After the bees had eaten all the poisoned food, great care was taken to see that they had plenty of nonpoisoned food. Bees soon die in such cages when deprived of food. The dead bees were counted and removed daily, and samples, each of 25 bees, were prepared for analysis. The results obtained are only comparative and are based on the average time required to kill all the bees tested, rather than on the absolute single lethal doses required to kill them. It was assumed that the bees ate equal quantities of the poisons, although this may never have been true; but even if bees do not eat equal quantities of the food given to them they have the habit of feeding one another, and consequently may on this account digest approximately equal quantities of food. In order to reduce the probable errors to a minimum, a large number of bees were used for each individual experiment.

EXPERIMENTS AND RESULTS

After the manner described in the preceding paragraph, 1 milligram of Paris green was fed to 500 bees; assuming that they shared it equally, each bee ate 0.0008 milligram of arsenic. These bees lived 1.5 days, on an average, with a minimum of 0.7 and a maximum of 2.7. Three samples of the dead bees yielded on analysis an average of 0.0006 milligram of arsenic per bee. A second lot of 500 bees was fed 0.5 milligram of Paris green in the poisoned candy, or 0.0004 milligram of arsenic per bee, and lived an average of 3.3 days, with a minimum of 0.6 and a maximum of 7 days. A third lot of 500 bees was fed 0.25 milligram of Paris green, or 0.0002 milligram of arsenic per bee, and lived an average of 8.3 days, with a minimum of 0.6 and a maximum of 18 days. On analysis, 3 samples of these bees were found to contain an average of slightly more than 0.0002 milligram of arsenic per bee. A fourth lot of 500 bees was in the same manner fed 0.15 milligram of Paris green, or a little more than 0.0001 milligram per bee, and lived from 1 to 13 days, with an average of 7. Two samples of these bees were found on analysis to contain an average of 0.0002 milligrams of arsenic per bee. As a control, 1,000 bees were fed nonpoisonous candy in confinement, and lived from 1.6 to 17.6 days, with an average of 9.8. Two samples of these were analyzed and found to contain 0.0001 milligram of metallic arsenic per bee. These experiments, with others of the same kind yet to be mentioned, are summarized in Table 7, with the results of analyses of dead bees and other data.

During this investigation many samples of bees, supposedly not poisoned, were analyzed. Some were found to contain as much as 0.0001 milligram of arsenic per bee, others a trace, and still others none at all. Since all animal tissue is said to carry at least a trace of arsenic, 0.0001 milligram each of metallic arsenic may here be considered the maximum quantity carried by control bees.

TABLE 7.—*Summary of results obtained by feeding arsenicals to bees in observation cages, 1914 and 1920*

Substances fed bees in each experiment	Number of bees fed	Average life of bees after eating the arsenical	Average quantity of arsenic (As)—		Number of bees analyzed
			Fed to each bee	Found per bee by analysis	
		Days	*Milligram*	*Milligram*	
Nonpoisoned candy (control)	1,000	9.8	Trace.	0.0001	50
Paris green in candy	500	.15	0.0008	.0006	75
Do	500	3.3	.0004	.0004	50
Do	500	8.3	.0002	.0002	75
Do	500	7.0	.0001	.0002	50
Acid lead arsenate in honey	500	6.0	.0010	.0009	125
Do	500	5.4	.0007	.0004	100
Do	500	5.0	.0005	.0004	100
Do	500	5.0	.0003	.0004	50
Arsenic acid in honey	100	5.46	.00049		
Acid lead arsenate in honey	100	5.71	.00049		
Sodium arsenate in honey	100	5.59	.00049		
Paris green in honey	100	5.59	.00057		
Do	100	5.76	.00057		
Do	100	5.77	.00057		
Zinc arsenite in honey	100	6.23	.00057		
Calcium arsenate in honey	100	6.91	.00049		
Poisoned pollen	500	6.9	.00004	.0003	100
Nonpoisoned pollen (control)	1,000	10.4	Trace.	Trace.	100

A fifth set of 500 bees was fed 5 milligrams of paste lead arsenate in honey, and lived from 0.3 to 21.3 days, with 6 days as an average. Providing they ate equal quantities of the poison, each one took 0.0010 milligram of metallic arsenic. When analyzed, 5 samples of them were found to contain an average per bee of 0.0009 milligram of metallic arsenic (Table 7). A sixth set of 500 bees was fed 3.5 milligrams of lead arsenate, or 0.0007 milligram each of arsenic, and lived from 2 to 10.7 days, with an average of 5.4. When analyzed, 4 samples of them contained only 0.0004 milligram of arsenic as an average per bee. A seventh set of 500 bees was fed 2.5 milligrams of lead arsenate, or 0.0005 milligram of arsenic each. They lived from 0.8 to 12 days, with 5 days as an average; when analyzed, 4 samples of them contained an average of about 0.0004 milligram of arsenic per bee. An eighth set of 500 bees was fed 1.5 milligrams of lead arsenate; if they ate equal quantities of the poison, each one took 0.0003 milligram of arsenic. When analyzed, each of 2 samples of these dead bees contained 0.0004 milligram of arsenic per bee.

For several days after the trees in the orchard at Winthrop, Me., had been sprayed in May and early June, 1914, poisoned pollen was stored in the hives. One of the bureau hives returned to Washington in June became depopulated August 1. To ascertain if the combs in this hive still contained poisoned pollen, 10 grams of the pollen were removed and were thoroughly mixed with 40 grams of honey. This mixture was then fed to 500 bees, as usual. They lived from 0.6 to 14.6 days, with 6.9 days as an average, whereas 1,000 control bees fed nonpoisoned pollen lived an average of 10.4 days (Table 7). When analyzed, 2 samples of pollen from the same combs were found to contain 2.57 parts and 1.19 parts of arsenic per million, the average being only one-fifty-first as much arsenic as was found in fresh pollen stored in the combs only a short time after it was collected from the sprayed trees. If the 500 bees ate equal quantities of the arsenic fed to them, each one took 0.00004 milligram of it, and this small quantity would not have noticeably affected them. When analyzed, 1 sample of the dead bees was found to contain 0.0001 milligram of arsenic per bee, each of 2 samples 0.0002 and 1 sample 0.0006 milligram, making an average of 0.0003 milligram of arsenic per bee (Table 7). It is thus seen that the small amount of arsenic still contained in this pollen was not equally eaten by the bees, and therefore the combs containing it should not be used again.

In June, 1920, eight arsenical spray mixtures were so prepared that they contained equal contents of arsenic acid or arsenious acid. A small portion of each of these was diluted 20 times, and 1 cubic centimeter of this dilution was thoroughly mixed with 4 cubic centimeters of honey in one of the small feeders. Providing the poisons were homogeneously mixed, each feeder contained 0.038 milligram of arsenic acid or arsenious acid; and, if the 50 bees in each feeder ate an equal share, each bee took nearly 0.0005 milligram of metallic arsenic when the arsenic acid or the arsenates were eaten, or 0.00057 milligram when the 3 mixtures of Paris green and the zinc arsenite were eaten. This series of tests was made a second time. Of these 8 arsenicals, the arsenic acid was most toxic (Table 7), but the sodium arsenate and 1 preparation of Paris green were close seconds, and the powdered acid lead arsenate third; the average life of bees after eating the 3 mixtures of Paris green is exactly the same as that of those eating the lead arsenate. The zinc arsenite and calcium arsenate seemed considerably less toxic, probably because the bees did not eat the mixtures containing them as readily as they did the others.

According to these figures, it appears that 0.0005 milligram of metallic arsenic is a conservative quantity to represent the minimum lethal dose per bee. Some of these results have already been published (*4, pp. 28, 48*).

In these experiments the behavior of the poisoned bees was carefully observed to determine whether it was similar to that of bees poisoned by arsenic in the fields. On the second day after having been poisoned many of these bees became more or less inactive and a few died; after that most of them were seldom seen eating; by the third day they were dying rapidly, their abdomens were much swollen, they could not fly, but could yet walk in a staggering manner, dragging their abdomens on the table. The visible effects of arsenic poisoning on them were similar to those of nicotine poisoning on bees already described by McIndoo (*9, pp. 91–92*), except that nicotine acts the more quickly, and its symptoms are the more pronounced, also that in cases of arsenic poisoning the abdomens are always more or less swollen, while this effect is rarely observed in cases of nicotine poisoning. Judging from the symptoms observed, bees fed arsenic seem to die of motor paralysis, although the paralysis may be only a secondary cause.

DISCUSSION OF LITERATURE

Only one of the many reports of the experiences of beekeepers will be cited here. This one, presented by Felt (*6*), states that several men sprayed their fruit trees with arsenicals while in full bloom, May 18 and 19, 1900. The first dead bees were observed on May 20. One beekeeper says:

> In front of each hive lay the full working force of the bees, some in clusters apparently dormant and others wriggling about as if in great agony. With the appearance of the sun there was a general movement among the bees in an effort to get as far away from the hive as possible. Those that had the strength would try to fly, but could only succeed in making 3 or 4 feet before they would drop to the ground.

The next day, May 21, a few hives were opened. Practically all of the field bees were gone, but a large quantity of brood and a few young bees were left. This beekeeper claims to have lost his entire apiary of 100 colonies, valued at $500.

According to the same report, another beekeeper claims to have lost between 80 and 95 per cent of the workers in his apiary of 95 colonies. Orchards were sprayed while in full bloom, 1½ miles north and the same distance northwest of a third beekeeper's apiary of 200 colonies, May 21 and 22. May 23, handfuls of dead and dying bees lay near the hives.

The following scientists have carried on experiments to determine the effects of arsenic on bees.

Brose (*1*) mixed London purple and Paris green in sirups in the same proportions in which these two insecticides are mixed with water for spray materials. When these poisoned sirups were fed to bees, they were not eagerly eaten. Some of the bees tested died 30 minutes after taking the poison, while others lived 4 hours. The chemical analyses of the sirups stored in the combs did not show arsenic, but traces of it were found in the bees killed by eating the sirups.

Cook (*2, p. 261*) claimed that bees are very susceptible to arsenicals sprayed on trees, but he did not ascertain this experimentally. The same author (*3*) experimented later with honeybees and several other insects. He confined them in cages and fed them mixtures composed of 1 pound or less of London purple to 200 gallons of water or sirup. Mixtures of 1 pound to 200 gallons were quickly fatal to honeybees.

Webster (*13*) sprayed a plum tree in full bloom with a mixture of Paris green and water; he then covered the tree with sheeting and mosquito netting and the ground with a canvas; a few hours later a hive of bees was placed inside the inclosure. The bees, instead of working normally, flew wildly about inside the inclosure, probably striking the foliage, flowers, sheeting, and netting; dead bees soon began to accumulate on the canvas. When a few of them were analyzed, arsenic was found; but its presence means nothing under the circumstances. The same author (*14*) performed experiments on a larger scale by spraying two apple trees in full bloom and a small orchard in which nearly all the petals had fallen. Several bees caught visiting the trees sprayed in full bloom were killed in a cyanide bottle, and parts of them were analyzed for arsenic; none was found in the hind legs with pollen and contents of the thoraces, but analyses of the contents of the abdomens, including the honey stomachs, revealed arsenic. Other bees, taken from the same trees, were killed, washed in dilute ammonia water, and analyzed, giving distinct traces of the poison. A few days after spraying the small orchard one of three colonies of bees near by suddenly became extinct and a second one greatly reduced in number of bees. From the totally depopulated colony Webster took dead bees, uncapped honey, and dead larvæ. When analyzed, the honey gave no evidence of arsenic, but the dead larvæ and the contents of the dead bees were found to contain it.

Woodworth (*15, pp. 111, 112*), reporting the work done by Volck at Watsonville, in the Pajaro Valley, Calif., states that a hive of bees was placed in the center of a 40-acre apple orchard, and then the trees were sprayed when just coming into full bloom. Analyses showed no arsenic in the new honey, but some in the pollen and dead bees, that in the latter approaching a toxic dose.

Holland (*8*) examined periodically in the laboratory many samples of dead bees and comb. A large percentage of the bees under observation died within two or three days. No cause for their deaths was known, except perhaps the spraying in the neighborhood. A small quantity of arsenic was found in 12 of 23 samples submitted for analysis. The detection of arsenic in the stored pollen was of special interest. Holland, using the approximate toxic dose for the horse, ox, sheep, and fowl as a basis, would call the toxic dose of arsenic for a honeybee about 0.005 milligram of arsenious oxide (As_2O_3) or 0.006 milligram of arsenic oxide (As_2O_5), which is 10 or 12 times the actual lethal dose arrived at as shown in the preceding pages.

Price (*12*) carried on experiments for two years in the field and laboratory. In the first year two apple trees were caged with cheesecloth. One was sprayed with lime-sulphur and the other dusted with flowers of sulfur and arsenate of lead, and a colony of bees was put within each inclosure and liberated. In the second year (1919) three trees were caged with wire screen, of which one was sprayed and one dusted, as in the first year, the third used as a control, and a normal colony of bees, with ample stores, placed in each of the three inclosures. Dead bees, collected from the cages, were counted and analyzed for arsenic in the laboratory. Three trees in full bloom in the open were also sprayed, while more than 200 were left unsprayed; live bees were counted on the sprayed blossoms and dead bees analyzed. In spite of the criticism that might be made in regard to the methods employed of confining bees with trees in large cages and of confining them singly in glass funnels when testing for fatal doses of arsenic, Price's conclusions are not far different from those reached in the present investigation. Less than 0.0005 milligram of arsenious oxide (As_2O_3) was found a fatal dose for a bee. The longevity of bees poisoned with arsenic was found to depend upon the size of the dose; some died within 1½ hours, others lived 5 or 6 hours, and most of those poisoned in the field died within 3 hours. Bees worked freely on sprayed trees in the open, even when there were unsprayed ones near by. The mortality of the bees in the control cage was 19 per cent, as compared with 69 per cent in the cages containing the trees sprayed with lime-sulphur and lead arsenate, and 48 per cent in the cage containing the tree dusted with sulphur and lead arsenate.

Doane (*5*) sprayed an apple tree, almost in full bloom, and then covered it with a cage. Two days later he placed a colony of bees inside this cage, and the following morning, while the bees were confined in their hive, the tree was again sprayed. He claims that these bees worked in an apparently normal way and were not injured by the arsenical spray material. This experiment was repeated, using another tree and another hive of bees, and similar results were obtained. His chemist reported for the small number of bees collected and analyzed that the dead bees collected in the cage at the end of the first experiment contained 0.0025 milligram, those collected alive during the first experiment 0.002 milligram, and those found dead during the second experiment 0.0006 milligram, respectively, of arsenic per bee. According to the results obtained by the present writers, less than these amounts of arsenic would, if eaten, have killed the bees. The next spring Doane repeated his experiments on a slightly larger scale, and finally concluded that spraying in full bloom under California conditions is not injurious to honeybees.

Merrill (*11*), while not repeating Doane's experiments, severely criticizes his methods, and compares his results with those obtained by Price, concluding that the latter were more likely to be correct.

SUMMARY

In the preliminary experiments conducted at Winchester, Va., 1914, three small, more or less isolated orchards were selected, and three strong colonies of bees were installed in each orchard. Twenty-five trees were sprayed in each orchard, the first 25 with a mixture of Paris green, the second 25 with a mixture of paste lead arsenate, and the third 25 with a mixture of paste lead arsenate and lime-sulphur. The following results were obtained: (1) Bees work equally as well on trees sprayed in full bloom as on unsprayed ones; (2) they do not fly away from the sprayed orchard very much if the orchard is well isolated; (3) they are slightly affected when a small orchard is sprayed in full bloom, a conclusion supported by observations and by the analyses of the samples of bees collected; and (4) of the three mixtures named, the arsenate-lime-sulphur mixture was found the most satisfactory for experimental purposes.

In the more extended experiments conducted at Winthrop, Me., 1914, a large isolated commercial orchard, consisting of 700 apple trees and almost ideally located, was selected, and 10 colonies of bees were installed in it on May 29 and 30. On May 25 and 26 all of these trees, nearly in full bloom, were sprayed with a mixture of paste lead arsenate and lime-sulphur; on May 28 and 29, 310 of them, now in full bloom, were again sprayed with the same mixture; and on June 3, 5, and 6 the entire orchard was again sprayed.

The unusual mortality of the bees at Winthrop was first noticeable the second day after they had had access to the sprayed flowers, and on the third day after they had been poisoned the damage was unmistakable. This heavy mortality continued as long as the sprayed flowers lasted, and in some of the colonies until all the bees had died. The symptoms of arsenic poisoning were very pronounced. In the early stage the adult bees became sluggish and soon neglected their duties, so that the brood apparently died of starvation; later their abdomens became greatly swollen, being filled with a yellowish watery liquid; still later the legs and wings became paralyzed; and finally the bees died in a state of coma. Not one of the 10 colonies used was killed outright, but in a comparatively short time 5 of them were entirely depopulated, while the other 5 were rendered more or less weak. It was thus ascertained that spraying fruit trees in full bloom under certain conditions is very injurious to bees.

The results obtained from the chemical analyses of the samples collected at Winchester and Winthrop agree with the field observations. Arsenic was found on all parts of the sprayed flowers, particularly on the pollen, and in most of the samples of the adult bees analyzed, but none was found in the samples of dead pupæ and larvæ. Of 12 samples of partially ripened honey analyzed only 1 contained arsenic, and that merely a trace. Dr. E. E. Tyzzer, of the Harvard Medical School, reports by correspondence that during the spring of 1920 and that of 1921 he experimented along similar lines. He says:

> I have had analyzed two samples of unripe honey, gathered during the spraying of fruit trees in full bloom, and found that both showed a very slight trace of arsenic. A sample of "honeydew" saved from a previous year showed 0.00045 per cent of arsenic, and another of ripe honey of good quality showed no trace of arsenic.

It thus seems that the bees obtained most of the arsenic from the pollen, although most of the nectaries analyzed contained arsenic. We should expect the honey to be poisoned, but so far only traces of arsenic have been found in it (see Table 1 for details).

The preceding results were obtained by daily observations, the use of several dead-bee traps, and analyses of the samples collected. One of these traps was installed in front of each hive selected for this purpose; it caught most of the bees that died in the hive, but their number was only about 2.5 per cent of the total mortality, the other 97.5 per cent dying in the field. When the mortality was heavy these traps were fairly satisfactory, for it is believed that the number of dead bees caught by them, before and after the spraying, represented a true mortality index of the bees tested. Because of the difficulties encountered in this kind of investigation, particularly when bees are poisoned but little or not at all, a more reliable method was introduced, in which all the interfering factors were either controlled or eliminated. Briefly stated, this method consisted of the following points: (1) The maturing brood was removed from six strong colonies, and by closely following a special procedure while weighing the hives with their contents on the platform scales the daily loss or gain in ounces of the adult bees were obtained independently of the weight of the stores, which were an interfering and variable factor; (2) an undisturbed colony was kept on these scales and was weighed hourly each day; (3) all the adult bees and the maturing brood in two other colonies were weighed three times on these scales, the weight of the stores being eliminated by following the special procedure just mentioned; (4) 10 outgoing and 10 returning field bees caught at the hive entrances were weighed on the chemical balances daily, every hour the bees were working; and (5) daily weather observations were made and recorded. In 1915 the writers worked separately, but thereafter together, using both methods.

Because of unfavorable weather no definite and conclusive results were obtained at Roswell, N. Mex., Benton Harbor, Mich., and Winchester, Va., in 1915. Most of the samples of dead bees collected at Roswell contained arsenic in very small quantities, but no symptoms of arsenic poisoning were noticed, except in two hives, which contained poisoned pollen collected from cover crops the preceding year. The trees at Benton Harbor bloomed irregularly, so that their blossoms were sprayed in all stages, thus causing a light mortality among the bees, and most of the samples of dead bees contained small quantities of arsenic. There did not seem to be any unusual mortality among the bees at Winchester, and not one of the 21 samples collected contained arsenic. (See Tables 2 and 6 for weight records at Winchester.)

The experiments at Winchester, Va., in 1916, were conducted in three apiaries, surrounded by large commercial orchards. The first apiary consisted of 70 colonies, the second of 40, and the third of 5. For the weighing experiments the first and third apiaries were used. From the time the trees were almost in full bloom until all the petals had dropped, the weather was almost ideal, and the bees worked normally. When the trees were sprayed nearly all the petals had fallen, and by this time the bees had almost ceased visiting the trees. The results from the various sources did not indicate that the bees tested had been poisoned, although 17 of the 28 samples collected contained very small quantities of arsenic, too small to have been fatal to bees. (See Tables 3 and 6 for weight records.)

The experiments at Fennville, Mich., in 1916, were also conducted in three apiaries, surrounded by commercial orchards. The first apiary consisted of 12 colonies, the second of 14, and the third of 30. For the weighing experiments 6 colonies in the first apiary were used. During the entire period of the experiments the weather was almost ideal, the blossoms secreted more abundantly than they did at Winchester, and the bees worked well in the trees. When the trees were sprayed the bees had almost ceased visiting them and practically all the petals had fallen. The results taken together indicated that the bees tested had not been poisoned, although again 17 of the 28 samples collected contained small quantities of arsenic, too little to have been fatal to bees. (See Tables 4 and 6 for weight records.)

In regard to the spraying of fruit trees at the customary time, when 90 per cent or more of the petals had fallen, at Winchester, Va., and Fennville, Mich., in 1916, under the most certain conditions, which are usually rare, it appears for the following reasons that the bees tested were not injured: (1) The old adult bees died more rapidly before than after spraying was begun, as was shown both by the daily weight records and by the number of dead bees counted in the bee traps. (2) The gain in weight, after the spraying was begun, of all the old and young adult bees in two colonies tested was equal to the gain before the spraying was begun. (3) The results obtained from the chemical analyses of the samples of dead bees, collected after the spraying was begun, did not indicate that the bees had died of arsenic poisoning. And (4) no symptoms of arsenic poisoning were noticed among the bees under observation.

The experiments at Winchester and Fennville were repeated on a small scale in 1917 at Drummond, Md., where the bee culture laboratory of this bureau was then located. Eleven apple trees were sprayed when about 90 per cent of the petals had fallen and when bees were to some extent still visiting these blossoms. Of the 40 samples of dead bees collected and analyzed only 3 showed the presence of arsenic, and these samples came from colonies which apparently had been suffering for some time from bee paralysis. No arsenic was found in samples of pollen taken from these hives. The other results obtained agree with those obtained at Winchester and Fennville in 1916. (See Tables 5 and 6 for weight records at Drummond.)

Some of the samples of dead bees collected when the trees were sprayed in full bloom contained on an average as much as 0.0004 milligram of arsenic (As) per bee. Of course, some of the individual bees contained less and some more than this quantity, yet it was not definitely known whether that quantity had actually killed them. A series of laboratory experiments in which definite quantities of several arsenicals were fed to bees decided this question in the affirmative, established the minimum fatal dosage of arsenic (As) per bee to be about 0.0004 or 0.0005 milligram, and showed that the symptoms of arsenic poisoning of bees in confinement were like those of the bees poisoned in the orchards. (See Table 7 for details.)

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